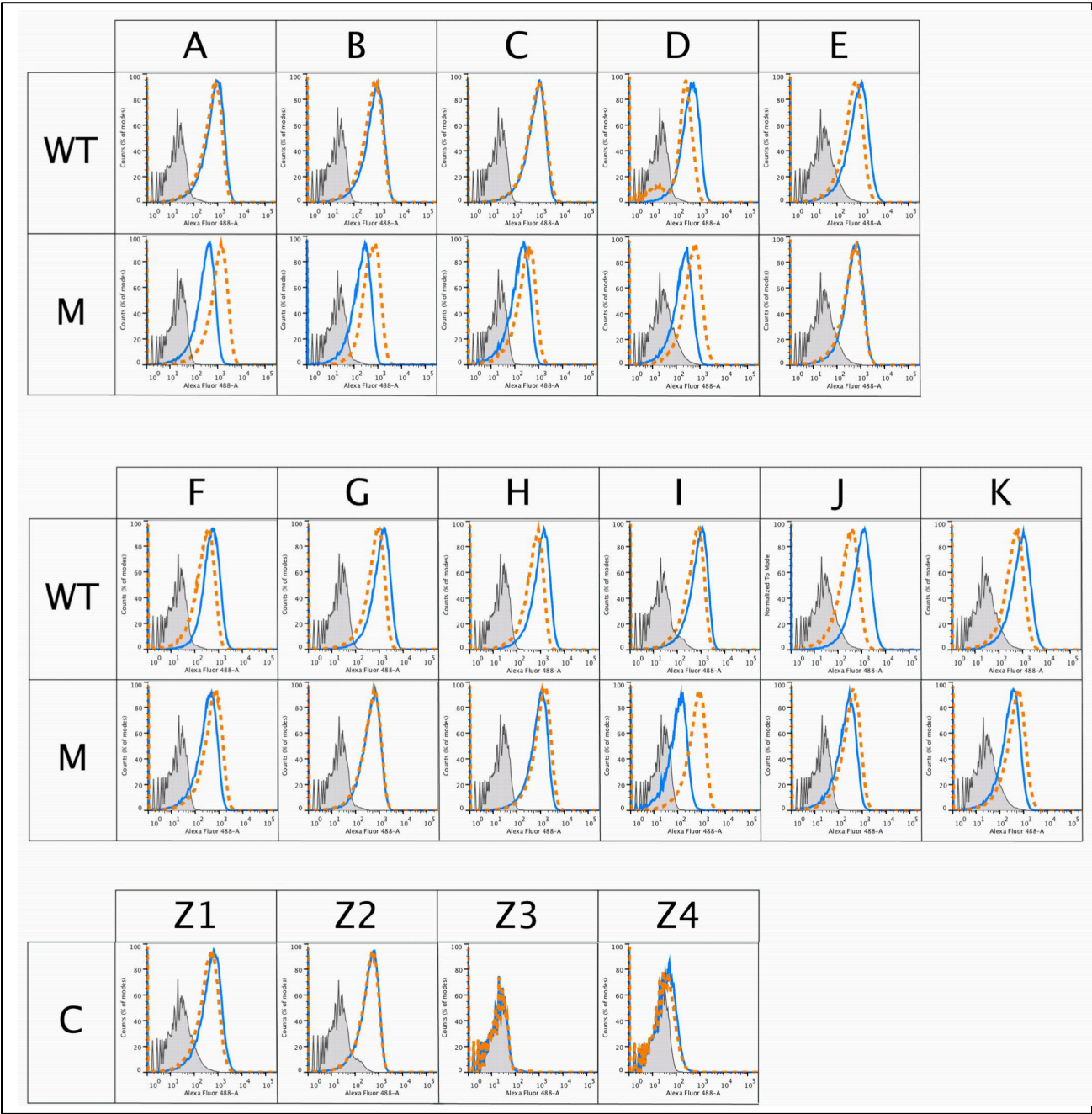
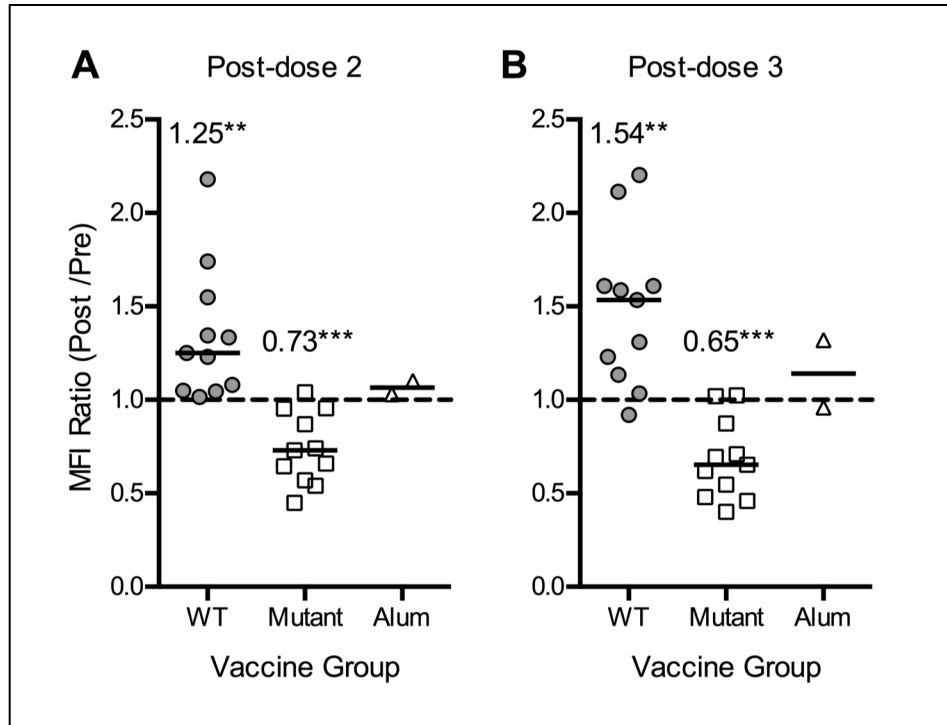


Supplemental Figure S1



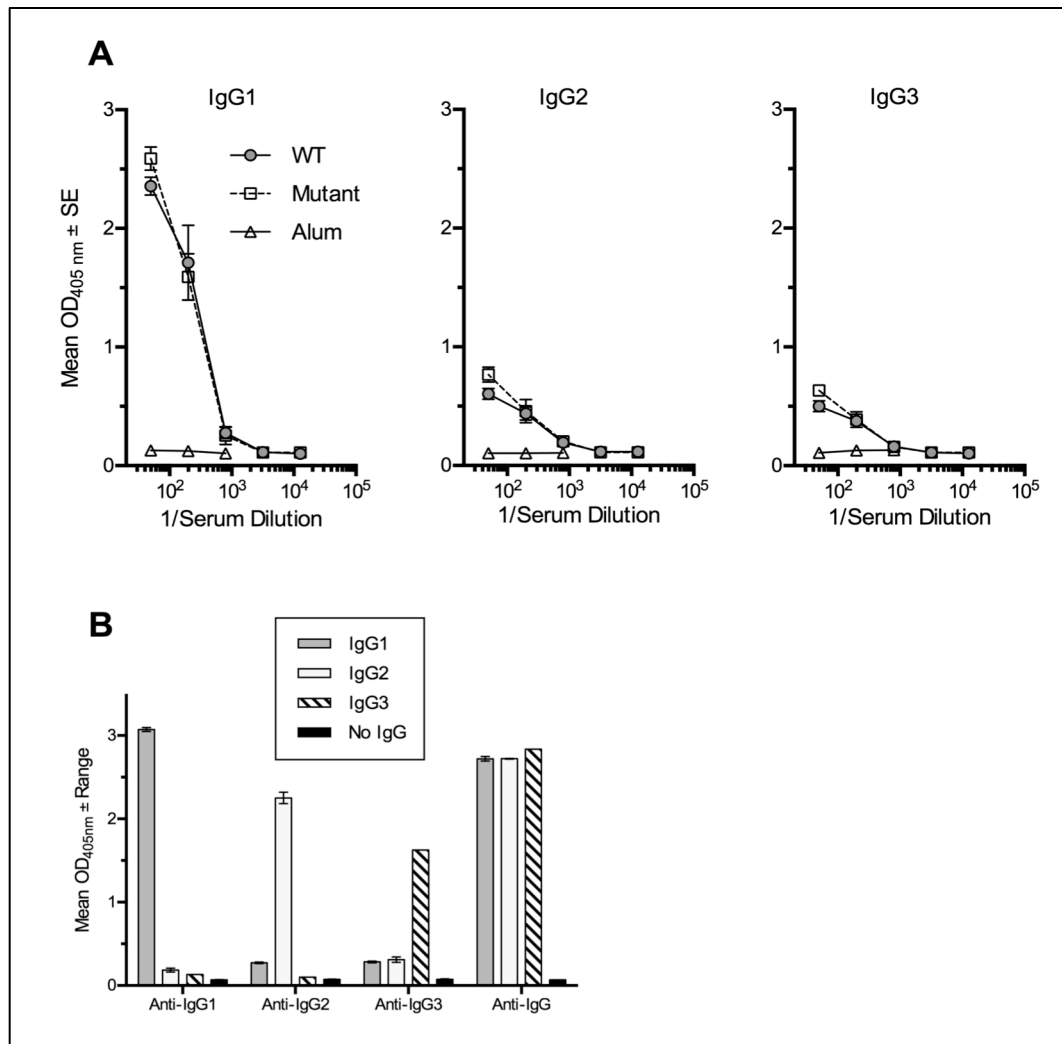
Supplemental Figure S1. The effect of FHbp immunization on binding of macaque serum FH to meningococci (strain H44/76) as measured by flow cytometry. Each histogram shows data for pre-dose 1 and 3 weeks post-dose 3 sera from an individual animal. All sera were tested at a 1:150 dilution. Gray filled areas represent bacteria not incubated with serum. Dotted orange lines represent binding of FH when the bacteria were incubated with pre-immunization sera and solid blue lines represent FH binding when the bacteria were incubated with post-immunization sera. Letters A to K refer to the pairs of macaques matched at the time of dose 1, with one animal was assigned to the wild-type (WT) FHbp antigen immunization group and the other assigned to the mutant (M) FHbp antigen group. Z1, 2, 3 and 4 refer to four negative control animals (C), each immunized with aluminum hydroxide alone (Z1 and Z2 have high FH binding to FHbp phenotypes, as have all of the animals given the FHbp vaccines, and Z3 and Z4 have low FH binding phenotypes). Six of the 11 animals in the mutant FHbp group (animals A, B, C, D, F and I) had lower FH binding in post-immunization serum than in pre-immunization serum (i.e. inhibition), compared to 0 of 11 animals in the WT FHbp group ($p=0.01$ by Fisher Exact test). Note also that none of the animals in the mutant vaccine group had higher binding of FH in post-immunization serum than pre-immunization serum whereas several animals in the WT FHbp group had enhancement of FH binding in post-immunization serum (for example, animals D and J). The respective results were confirmed in an independent assay. The median fluorescence intensity (MFI) values for each individual animal before and after vaccination as measured in replicate assays are shown in **Figure 4D** and **E** of text.

Supplemental Figure S2



Supplemental Figure S2. Effect of FHbp vaccination on binding of serum FH to meningococci (strain H44/76). **(A)** The graph depicts the ratio of FH binding in post-dose 2 to pre-dose 1 sera as determined by flow cytometry. Each symbol represents the ratio of the median fluorescence intensity (MFI) of FH binding (post-dose 2/pre-dose 1) for an individual animal as determined in two to three independent assays. The horizontal lines indicate the median value of each group. Ratios ≥ 1 signify no inhibition, or enhanced FH binding. Ratios < 1 indicate inhibition of FH binding. The median ratios in the two vaccine groups were significantly different from 1.0 ($p \leq 0.01$ by Wilcoxon signed-rank test). **(B)** For comparison, the data from Figure 4F depicting the ratios of post-dose 3/pre-dose 1 sera are shown (see legend to Figure 4F).

Supplemental Figure S3



Supplemental Figure S3. Serum IgG subclass-specific anti-FHbp antibody responses. IgG subclass-specific anti-FHbp titers were measured by ELISA using mouse mAbs specific for rhesus macaque IgG1, IgG2 and IgG3 (see Methods). To control for differences in overall IgG titers, data are shown for the six macaques in the WT FHbp group with the highest serum IgG anti-FHbp titers, and the six macaques in the mutant FHbp vaccine group with the lowest serum anti-FHbp titers (See **Figure 5** of body of article). **A**, Mean optical density (OD_{405nm}) ± SE for IgG1, IgG2 and IgG3 binding to FHbp in each vaccine group at different serum dilutions (N=6 macaques in each FHbp vaccinated group and N=4 negative control macaques immunized with aluminum hydroxide without an antigen). The respective subclass responses of the two FHbp groups were not significantly different ($p>0.05$). **B**, Mean optical density (OD_{405nm}) ± range of duplicate determinations for binding of a 1:1000 dilution of each of the mouse anti-rhesus macaque IgG subclasses and total IgG antibodies to microtiter wells coated with 10 µg/ml of recombinant rhesus macaque IgG1, IgG2 or IgG3.